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Isolation, characterization and generation of functional macrophages from human cord blood-derived cd34+ hematopoietic cells

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BACKGROUND: Umbilical cord blood (UCB) has become an alternative source of hematopoietic progenitor cells for transplantation. The aim of this study was to test the effectiveness of some modifications of human hematopoietic stem cells isolation protocols with the intention of improving the output and viability of CD34+ cells and progenitor subpopulations progeny that can be obtained from a sample of human umbilical cord blood and keeping the ability of long-term self-renewal and the capacity to give rise to one or more types of differentiated progeny.

METHODS: Human cord blood samples were transformed to buffy coat prior to the isolation of HSCs which was performed by two steps involving CD34 pre-enrichment using human cord blood CD34 positive selection kit and an immunomagnetic cell separation, targeting CD34 surface antigen. CD34+ cells were immunophenotyped by four-color fluorescence, using a large panel of monoclonal antibodies (CD34/PE, CD45/FITC, CD38/APC, CD33/Per-Cy, HLA-DR/PE, CD117/APC, CD123/Per-Cy, CD105-FITC, CD56/PE, CD14/Per-Cy, CD19/Per-Cy and CD3/APC) recognizing different lineage or activation antigens. The isolated CD34+ cells were also studied for proliferation and differentiation in liquid cultures in the presence of Flt3L, SCF, IL-3, IL-6 and M-CSF. Using differing combinations of growth factors the effect on cell proliferation and differentiation was determined. The macrophage-like phenotype was confirmed by analyses of surface markers, histo-morphology and phagocytosis.

RESULTS: Our results showed that the percentage of CD34+ cells in whole human cord blood samples was 0.02% of total cells. After isolation by two-step, combining CD34 pre-enrichment and immunomagnetic isolation, the frequency of CD34+ stem cells represented 0.65% among total MNCs and 83.53% among total isolated cells. This isolation leaded to a purity of over 95% and viability of 98.60%. In addition, we found that the percentage of CD34+ cells which are CD45+ was 83.53%, whereas CD34+CD38- cells comprised 21.70%. About 70.85% of isolated CD34+ cells were characterized by the absence of human leukocyte antigen-DR (HLA-DR). Concerning the CD117, CD33, CD123 and CD105 antigens which characterize true stem cells, we found a high expression percentage among isolated HUCB CD34+ cells (81.26%, 57.14% 47.45%, 58.52% for CD117, CD33, CD123 and CD105, respectively), while a very small number displayed markers of advanced myeloid commitment, such as CD14 (Myeloid lineage, 0.7%) and CD56 (NK-cell lineage, 4.48%), or those of lymphoid differentiation: CD3 (T-cell lineage, 5.22%), and CD19 (B-cell lineage, 1.76%). We also demonstrated that these isolated CD34+ cells can be expanded and differentiate; a homogeneous population of CD14-positive monocytes is produced. These hES monocytes have a distinct myeloid signature and are capable of differentiating into functional macrophages. These macrophages are phagocytic; they respond to leishmanial infection and express phenotypic markers of mature macrophages.

CONCLUSIONS: We conclude that our modified technique enabled us to obtain an important proportion of primitive hematopoietic progenitors, as suggested by the absence of HLA-DR and CD38, as well as the presence of CD117, CD33, CD123, and CD105 on their surface. These cells are recognized as having long-term reconstitution capacity within the human CD34+ cell population. Therefore, the production of fully functional Human CD34- hematopoietic stem cells has been successfully achieved.

Biography

Souad Al-Okla was an associate professor at Damascus University. I have a Bachelor's degree in Natural Sciences (Biochemistry), Diploma (DES) in Biology, Master in Immunology (all from Faculty of Sciences, Damascus University, Syria). In addition, I have Diplome d'Etudes Approfondies (DEA) in Molecular and Cellular Pharmacology and Ph.D. in Immunology (both from Faculty of pharmacy, University of Strasbourg II, France). I have an experience in teaching (12 years: Biology, Immunology, genetics and medical genetics) and in research (students of master and Doctorate) in faculties of Sciences, Medicine, Pharmacy, dentist and agriculture in public and private Universities in Damascus. My Actual job is researcher and Associate Professor at the Faculty of Sciences, Damascus University. Furthermore, I am the Head of IVF Lab in IVF and Sterility Unit in Obstetrics& Gynaecology Hospital at the Faculty of Medicine in Damascus University. Actually I am working as associate professor at Oman Medical College.

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